



Comparison of anther culture technique efficiency in the production of wheat and triticales double haploids

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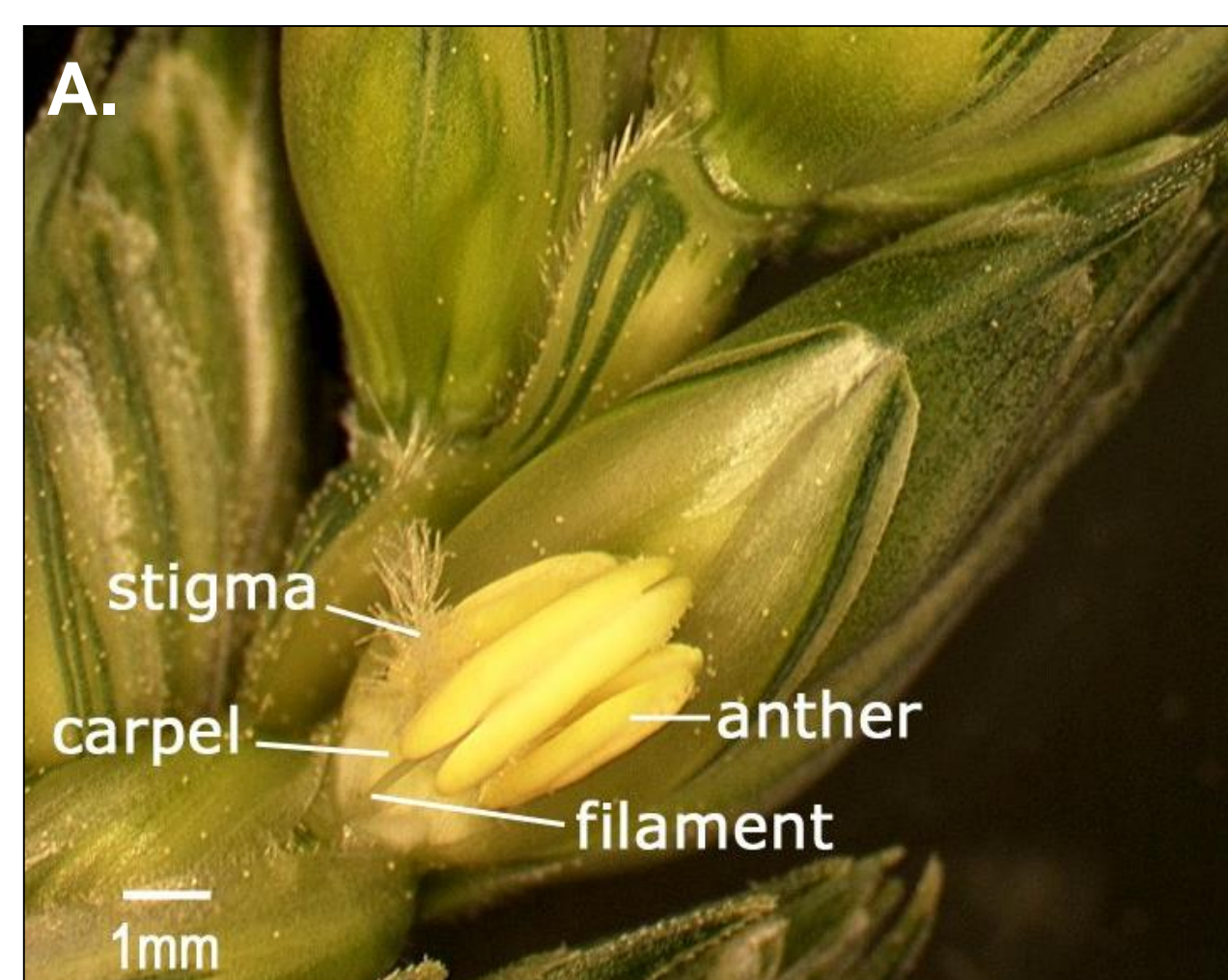
INTRODUCTION

In winter wheat and triticales species, doubled haploid (DH) production methods offer the quickest way for the production of homozygous lines. The latter capacity is of key importance in cereal crops improvement already for decades. By using elite lines as crossing parents, combined with the opportunity to select more efficiently agronomic traits in homozygous plants. DH breeding strategies have competitive advantages compared to conventional methods.

The use of anther culture in wheat and triticales breeding programs is limited by strong genotype specificity, low frequency of haploids and a high rate of albinism in regenerants. Despite these problems, the anther culture technique has been successfully used in breeding programs, resulting in release of new cultivars. Figure 1 is presenting isolation of wheat anthers.

The objective of the present study was to compare androgenesis efficiency in the production of spontaneous dihaploid (DH) lines from randomly selected heterozygous winter wheat and winter triticales genotypes.

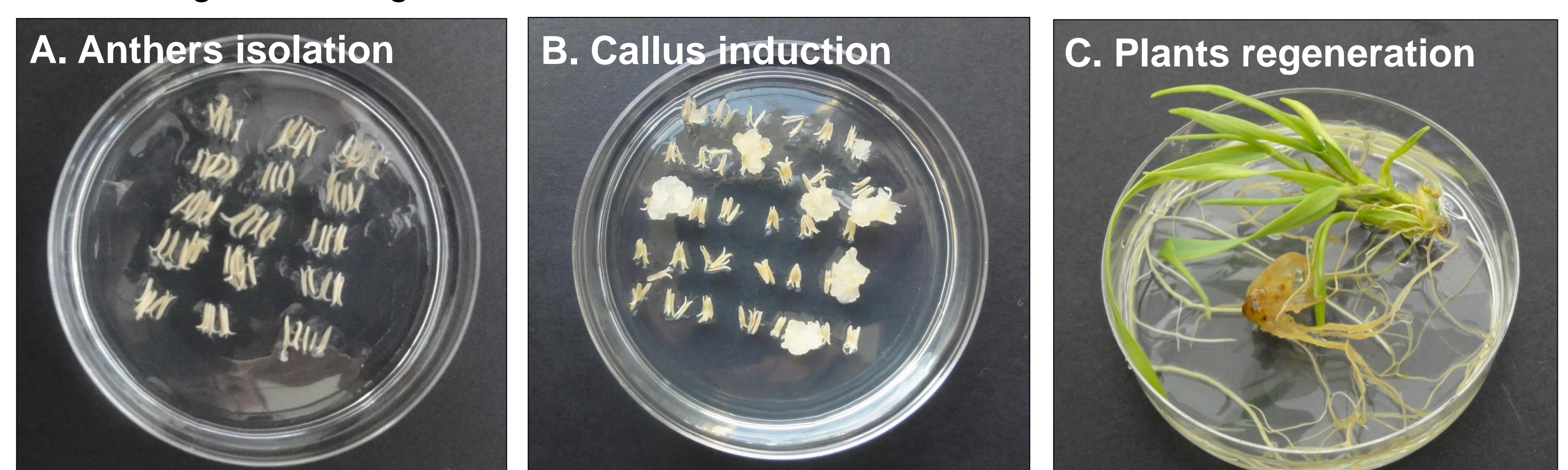
Figure 1.
A. Flower structure of wheat.
B. Wheat anthers isolation.



EXPERIMENTAL PROCEDURE

1. Growth of the donor plants under controlled conditions.
2. The spikes of each combination were used at the mid- or late uninuclear stage of microsporogenesis.
3. Pre-treatment of spikes in darkness ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for 21 days.
4. Spikes' sterilization with 70% ethanol and 5% sodium hypochlorite.
5. Isolation of anthers from spikes under aseptic conditions and transfer on the surface of induction media (Figure 2).
6. Incubation of plates with anthers in the dark ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for 6-8 weeks.
7. Transfer the explants with calli on regeneration medium.
8. Transfer of calli with green shoots to the rooting medium.
9. Analysis of the androgenesis efficiency.

Figure 2. Androgenesis diagram.



RESULTS

Androgenesis was induced during the treatment of each tested genotype. All of examined genotypes showed the ability to produce pollen calli. Our results demonstrated significantly higher efficiency of callus induction and plant regeneration by F1 hybrids of winter triticales. On average, 2.77 wheat calli and 19.41 triticales calli were produced per 100 cultured anthers. The green plants were regenerated for 16 wheat and 18 triticales genotypes. During the experiments, 449 plants were regenerated, of which 31 were winter wheat plants. Plant regeneration efficiency was observed 3.86 for wheat and 10.45 for triticales lines. The genotypes with a relatively high regeneration capability were: DH162 for wheat (7 plants were obtained) and DH124 for triticales (130 plants were received).

Table 2. Somatic embryogenesis efficiency for triticales and wheat

Wheat lines	No of anthers	No of EC	No of plants	% of EC	% of planlets
DH 158	410	19	1	4.63	5.26
DH 159	570	13	1	2.28	7.69
DH 160	1385	21	1	1.52	4.76
DH 161	469	15	-	3.20	-
DH 162	1046	13	7	1.24	53.85
DH 165	860	26	-	3.02	-
DH 166	1197	30	-	2.51	-
DH 167	599	10	-	1.67	-
DH 168	3178	91	-	2.86	-
DH 169	1980	46	1	2.32	2.17
DH 170	937	133	2	14.19	1.50
DH 171	1169	24	2	2.05	8.33
DH 172	2919	93	2	3.19	2.15
DH 173	1073	28	1	2.61	3.57
DH 174	920	10	-	1.09	-
DH 175	724	20	1	2.76	5.00
DH 176	1786	165	1	9.24	0.61
DH 177	1464	59	-	4.03	-
DH 178	3697	30	1	0.81	3.33
DH 179	4528	69	1	1.52	1.45
DH 180	3653	184	-	5.04	-
DH 181	3328	55	-	1.65	-
DH 182	4380	61	1	1.39	1.64
DH 183	2290	71	4	3.10	5.63
DH 184	3336	77	-	2.31	-
DH 185	1680	19	-	1.13	-
DH 187	3869	46	4	1.19	8.70
MEAN	55374	1432	31	277	3.86

Triticales lines	No of anthers	No of EC	No of plants	% of EC	% of planlets
DH 116	980	152	2	15.51	1.32
DH 117	2769	206	11	7.44	5.34
DH 120	1170	122	17	10.43	13.93
DH 121	2025	603	53	29.78	8.79
DH 122	1950	539	48	27.64	8.91
DH 124	1356	432	130	31.86	30.09
DH 126	360	132	33	36.67	25.00
DH 127	1774	240	29	13.53	12.08
DH 128	850	167	44	19.65	26.35
DH 130	90	22	1	24.44	4.55
DH 131	2170	154	16	7.10	10.39
DH 132	2591	155	1	5.98	0.65
DH 134	765	45	3	5.88	6.67
DH 141	110	36	10	32.73	27.78
DH 142	1141	181	10	15.86	5.52
DH 145	467	81	3	17.34	3.70
DH 152	93	37	6	39.78	16.22
DH 153	176	29	-	16.48	-
DH 154	200	57	1	28.50	1.75
DH 156	180	3	-	1.67	-
MEAN	21217	3393	418	19.41	10.45

PLANT MATERIAL

Twenty seven wheat lines and twenty winter triticales lines were used to select genotypes with a high regeneration capability (Table 1.).

Table 1. Wheat and triticales cultivars used for crossings

WHEAT CULTIVARS	TRITICALES CULTIVARS
Arkadia	Algoso
Astoria	Borowik
Bamberka	Borwo
Muza	Cyrkon
Ostroga	Meloman
Wydma	Tomko
	Panteon

SUMMARY

In conclusion, we have demonstrated significant effects of genotypes on callus induction and plant regeneration from anther cultures of wheat and triticales lines. We have selected a few genotypes with a high regeneration capacity. These plants can be used for future production of DH lines for breeding, transformation and other biotechnological studies. In the future work an effort will be undertaken to improve the androgenesis efficiency for wheat lines.

ACKNOWLEDGMENT This work was financially supported by Ministry of Agriculture and Rural Development of Poland.